MYOCARDIAL PERFUSION

Computational insights on coronary artery function

Collateral arteries may act as natural bypasses that reduce hypoperfusion after a coronary blockage. 3D imaging of neonatal and adult mouse hearts, plus human fetal and diseased adult hearts, is now used to computationally predict flow within the heart, and understand the cardioprotective role of collateral arteries in vivo.

David Mayerich and Joshua D. Wythe

Heart disease is the leading cause of mortality and morbidity worldwide[1–3]. Cardiomyocytes, the muscular working cells of the heart, have immense energetic and nutritive demands, which the coronary vasculature normally meets by constantly supplying oxygen, nutrients and hormones to the continuously pumping heart. However, blockage or occlusion of these vessels, as occurs in coronary artery disease, can lead to a heart attack or acute myocardial infarction, resulting in the death of potentially up to one billion myocytes distal to the infarcted region[4]. Unlike other species, myocytes in the adult human heart are considered terminally differentiated, with limited cell cycle activity. Thus, the irreversible loss of cardiomyocytes after myocardial infarction leads to the formation of permanent scars owing to extensive inflammation and compensatory fibrosis, which in turn results in cardiac stiffening and decreased contractility, and this adverse remodeling ultimately leads to heart failure.

Despite decades of work, the focus on repairing the heart via either exogenous or endogenous sources of stem or progenitor cell populations has yielded few, if any, tangible improvements[4]. Current approaches that focus on cardiomyocyte renewal — such as by stimulating endogenous cell cycle entry and directed reprogramming of existing non-myocyte populations — may hold more promise for mending the damaged heart[5]. However, coronary artery disease still produces an immense health and economic burden[2], and advances that promote myocyte renewal of ischemic hearts, as well as revascularization, are desperately needed[6].

In patients with ischemic disease, the main compensatory response to blocked or reduced blood flow is mediated by collateral vessels: natural bypasses that link major artery branches without intervening capillaries. These connections (anastomoses) partially attenuate hypoperfusion or ischemic injury after blockage of a coronary artery by generating a functional link, or bypass, between adjacent vessels that enables the reperfusion of tissues downstream of an occluded vessel. Notably, collaterals vessels are evident during gestation[7] and are present in individuals with no previous occlusion[8]. The extent of collaterals present affects clinical outcomes[9], as patients with extensive collaterals can survive blockage of native coronary arteries and show normal cardiac function. However, studies in neonatal mice, which possess a regenerative capacity to recover normal cardiac function after myocardial infarction, suggest that although collateral coronary arteries are normally not present[10], they do form after vascular occlusion via a variety of mechanisms, including arteriogenesis[11] and arterial assembly[12].

Despite these mechanistic breakthroughs, how structural parameters (such as hemodynamics) affect collateral formation after injury remain unknown owing to the inability to obtain accurate, direct measurements of collateral flow across an entire heart. Given the small size of the mouse heart, visualization of the coronary circulation has provided limited evidence on which to base solid conclusions about collateral formation and utilization after injury. This knowledge gap has been filled with ex vivo, static 2D imaging data after perfusion or casting, which poses considerable caveats. Accordingly, a better understanding of collateral growth in the healthy and injured mammalian heart is desperately needed.

In this issue of *Nature Cardiovascular Research*, Anbazhakan et al. combine whole-organ clearing and 3D imaging with computational modeling to predict perfusion in the neonatal and adult mouse heart[13] (Fig. 1). By exploiting recent innovations in tissue-clearing and whole-organ immunolabeling[14], the authors generated high-resolution, 3D confocal maps of the entire arterial tree within the developing and adult mouse heart. Acting as rigorous cardiac cartographers, they went on to quantify the type, number and size of collateral vessels in both neonates and adults before and after injury.

As an initial step, using iDISCO and light-sheet microscopy to characterize collateral bridges in neonates and adult mouse hearts after myocardial infarction, they established the functionality of these collaterals by perfusing with fluorescent-conjugated antibodies before euthanizing the mice, confirming that these vessels were patent and carry blood flow. Their initial characterization showed that most collateral bridges in neonates were located at the edge of the infarcted area, and half of all collaterals linked healthy tissue to infarcted, necrotic regions. A similar pattern was observed after myocardial infarction in the adult mouse heart.

They then used computational fluid dynamics (CFD) to estimate blood flow in the coronary tree of the neonatal and adult mouse heart under varying parameters (for example, number, size and location of collateral vessels) to understand their effect on blood flow after injury. The authors undertook the herculean task of manually segmenting more than 500 vessels, having vascular anatomists independently quantify vessel positions and diameters to compensate for user variability in the segmentation. The resulting 3D vascular models underwent CFD modeling, which integrated known laws of fluid physics with experimentally determined flow data collected in vivo in other larger animal models. The results were scaled to match the mouse vasculature, creating high-resolution models of coronary circulation and perfusion. These models allowed the researchers to generate virtual occlusions (or blockages) and alter vessel patterns (changing both number, size and location)

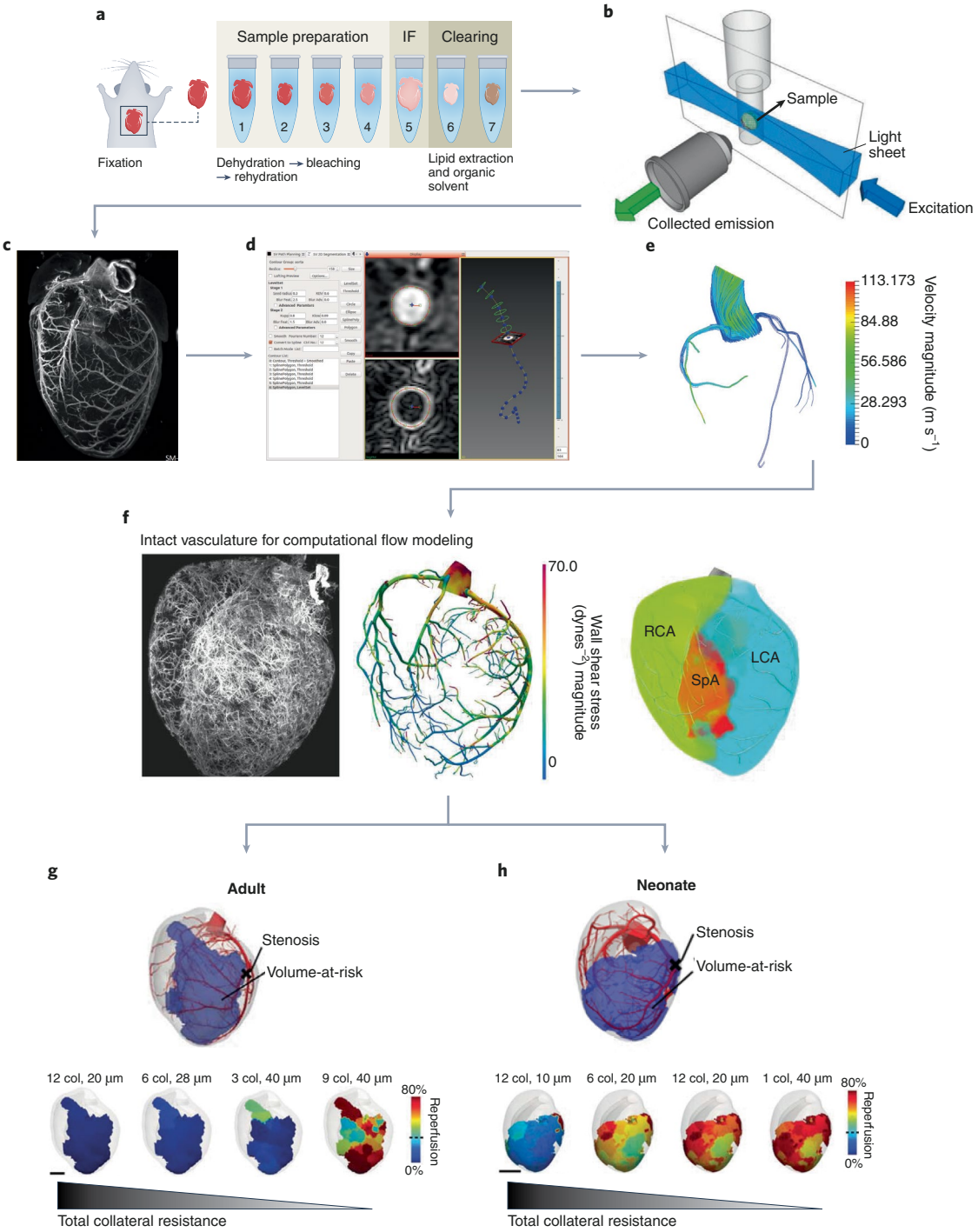

Fig. 1 | Pipeline for image acquisition and computational prediction of flow dynamics in collaterals of the mouse heart after myocardial infarction. **a**, **b**, Mouse hearts are immunostained to fluorescently label the coronary arteries, then cleared using iDISCO (**a**) and imaged using light-sheet fluorescent microscopy (**b**). **c**, **d**, The resulting images (**c**) are manually segmented to construct the vessels and validated by several independent users (**d**). **e**, **f**, Fluid flow through these vascular networks is modeled using the computational fluid dynamics package SimVascular (**e**) to produce coronary artery models (**f**). **g**, **h**, Model parameters (such as vessel number, diameter and location) are changed to test hypotheses in the adult (**g**) and neonatal (**h**) mouse heart after myocardial infarction. IF, immunofluorescence; LCA, left coronary artery; RCA, right coronary artery; SpA, septal artery. Panels adapted with permission from: **b**, ref. [16], The Optical Society; **g**,**h**, ref. [13], Springer Nature Ltd. Panels reproduced with permission from: **c**,**f**, https:go.nature.com/3orhze0, Kristy Red-Horse; **d**,**e**, https://simvascular.github.io/index.html, Alison Lesley Marsden.

to determine how various configurations of collaterals affected blood flow after injury.

Notably, they found that collaterals that form after injury in the adult heart perform poorly, doing little to restore flow and reperfuse distal tissues. Administration of the potent chemokine CXCL12 — which they previously demonstrated enhanced recovery after myocardial infarction in mice and induced larger diameter collaterals[12] — led to increased, but sub-optimal reperfusion. However, increasing the diameter of these vessels was predicted to reduce resistance and enhance their function. By contrast, CFD modeling demonstrated that native collaterals formed after injury in the regenerative neonatal mouse heart are superior in terms of restoring flow. In fact, even one large (40-µm diameter) vessel was predicted to provide massive perfusion recovery in neonates.

Digging deeper into their model, the authors modulated the degree of stenosis and the percentage of flow recovery, which confirmed that identical collateral configurations composed of vessels of the same size function better in the neonatal heart. The authors postulated that this is probably due to a more effective distribution network and smaller heart size in neonates. They then asked whether altering the placement of collaterals could improve recovery in adults. Placing virtual vessels more proximal in the coronary tree almost doubled flow recovery and tripled the perfusion territory. This suggested that fewer larger collaterals are more beneficial and cardioprotective than more numerous smaller collaterals that probably distribute flow at a level that is insufficient to promote recovery.

They then used immunostaining and imaging to confirm the presence of extensive collaterals within the fetal human heart[7], which is in stark contrast to their absence in the developing mouse heart. These fetal imaging studies were complemented by adult angiogram data, which detected only between one and four collaterals in patients with chronic arterial occlusions. CFD modeling predicted that human fetal collaterals are too small to noticeably restore flow, and the functionality of collaterals in patients (determined by hemodynamic capacitance correlated with their diameter and shape) lies somewhere between neonatal and adult mouse collateral vessels. However, vessel diameters from patient data calculated using 2D angiogram projections are probably inaccurate, which would affect modeling, and angiograms only highlight a subset of collaterals.

These computational modeling studies suggest that supplying fewer, larger diameter arterial connections may ameliorate a proximal stenosis. Future gains in automated segmentation, using packages that leverage machine learning such as TubeMap or ClearMap[15], will undoubtedly accelerate our understanding of how alteration of the collateral coronary network can positively affect recovery after myocardial infarction. The daunting question remains as to what determines coronary artery patterning and the origin of collateral vessel sprouting. The authors' data agree with the hypothesis that hypoxia initiates collateral formation, as bridges were formed within or near infarcted regions. However, tissue stiffness and hemodynamic feedback (for example, sheer stress from oscillatory flow, vessel shape, diameter and thus resistance) also likely impact collateral formation. This elegant study lays the foundational groundwork for investigations using larger animal models in which flow dynamics and pressure can be accurately measured and correlated to whole-heart 3D imaging for guiding future therapies targeting collateral vascularization after myocardial infarction. In keeping with the ethos of *Nature Cardiovascular Research*, and to facilitate studies by other groups, the authors supplied all custom code on Github (https://github.com/StanfordCBCL/Collateral), the modeling software is freely available (https://simvascular.github.io/) and all datasets from the manuscript are publicly available (https://doi.org/10.25740/qk058jq2233; https://www.vascularmodel.com/repository.html). ❐

David Mayerich[1] and Joshua D. Wythe[2,3,4] ✉

[1]*Department of Electrical and Computer Engineering, University of Houston, Houston, TX, USA.* [2]*Department of Integrative Physiology, Baylor College of Medicine, Houston, TX, USA.* [3]*Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX, USA.* [4]*Center for Organ Renewal and Repair, Baylor College of Medicine, Houston, TX, USA.*

✉*e-mail: wythe@bcm.edu*

Published online: 12 August 2022
https://doi.org/10.1038/s44161-022-00115-8

References

1. Timmis, A. et al. *Eur. Heart J.* **43**, 716–799 (2022).
2. Virani, S. S. et al. *Circulation* **143**, e254–e743 (2021).
3. Zhao, D. *JACC Asia* **1**, 1–13 (2021).
4. Lazar, E. et al. *Eur. Heart J.* **38**, 2333–2342 (2017).
5. Garbern, J. C. et al. *Dev. Cell* **57**, 424–439 (2022).
6. Tian, X. et al. *J. Mol. Cell. Cardiol.* **167**, 67–82 (2022).
7. Cortis, B. S. et al. *Cardiologia* **43**, 77–81 (1998).
8. Wustmann, K. et al. *Circulation* **107**, 2213–2220 (2003).
9. Seiler, C. *Eur. J. Clin. Invest.* **40**, 465–476 (2010).
10. Zhang, H. et al. *J. Mol. Cell. Cardiol.* **87**, 4–16 (2015).
11. He, L. et al. *Cardiovasc. Res.* **109**, 419–430 (2016).
12. Das, S. et al. *Cell* **176**, 1128–1142 (2019).
13. Anbazhakan, S. et al. *Nat. Cardiovas. Res.* https://doi.org/10.1038/s44161-022-00114-9 (2022).
14. Renier, N. et al. *Cell* **159**, 896–910 (2014).
15. Kirst, C. et al. *Cell* **180**, 780–795 (2020).
16. Olarte, O. E., Andilla, J., Gualda, E. J. & Loza-Alvarez, P. *Adv. Opt. Photonics* **10**, 111–179 (2018).

Competing interests

D.M. is a founder and stakeholder of Swift Front.